Title

Genetic and clonal structures of the tree species *Tilia cordata* Mill. in remnants of ancient forests in Denmark.

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Abstract

The insect-pollinated forest tree *Tilia cordata* Mill. grows today in small fragmented populations in Denmark and other western European countries but was, in pre-historic times, a dominating species and is considered an indicator species for ancient forest. The species is known to propagate both sexually and vegetatively, forming clonal groups. Few studies have been made on the species' population genetics and on how clonality affects the population structure. The aim of this study is to evaluate the Danish gene pool by estimating genetic diversity and differentiation, as well as through exhaustive sampling describe clonal structures in some of the populations. Genetic analysis was carried out using nine nuclear microsatellite markers in nine populations, of which four were exhaustively or partly exhaustively sampled. The markers showed a high degree of genetic diversity but low differentiation between populations, with no geographic related structure. Clonal structures were found in eight out of the nine populations. In the exhaustively sampled populations, recruitment strategies included both sexual and clonal reproduction with indications that clonality may be enhanced by management and other disturbances.

Keywords: clonality, clonal reproduction, heterozygosity, population genetics, *Tilia cordata*

Introduction

Tilia cordata mill. (small-leaved lime) is a tree species indigenous to Denmark where it has a highly scattered and fragmented distribution and is considered a marginal forest tree species (Lawesson 2004). Further, it is known as an indicator species for ancient forests in northern Europe, with ancient being defined as forests where the forest cover has been persistent but often under management schemes, such as coppicing. It should therefore not be mistaken for virgin forest, untouched by man (Hermy, Honnay, & Firbank, 1999; Lawesson, 2004; Rackham, 2008).

The genus *Tilia* consists of 23 forest tree species, all insect pollinated, mainly found in the temperate zone in the northern hemisphere, with the only exception of two species found in tropical Vietnam and Mexico (Pigott, 2012). Four species of *Tilia* are native to Europe (*T. cordata*, *T. platyphyllos*, *T. dasystyla* and *T. tomentosa*) and of these, only *T. cordata* and *T. platyphyllos* grow in northern Europe. *Tilia cordata,* with the most widespread distribution, reaches from the British Isles in the west to Siberia in the east, from Norway in the north to northern Greece in the south (Pigott, 2012). After the icecaps from the last glacial maximum had retreated, *Tilia* was the dominant species in the forests of Northern and Western Europe during the warm Atlantic period ca. 7000 to 4000 before present, a partly overlapping period in Denmark is known as the "*Tilia* period" and spans from 9000 to 2500 years before present (Rehfeldt, 1994). Around 4000 years ago, this started to change as *Fagus sylvatica* began to establish and take over as the dominating tree. This transition in the forest composition is believed to be a result of a mix of natural and anthropogenic processes (Turner, 1962).

In Denmark, *Tilia* spp. also used to be a dominant species, but is today only found in a few scattered populations across the country and mainly with few individuals in each population. Lawesson (2004) only found *T. cordata* in 77 forests out of 1100 surveyed Danish forests and *T. platyphyllos* even more rarely. Of the 77 forests, *T. cordata* was only abundant in three of them, and in 53 of the forests, fewer than 50 individuals were observed. Pigott (1991) reports that the species rarely re-enters a forest once removed, which is the prime reason that it can be used as an indicator for ancient forest (Hermy et al., 1999; Rackham, 2008). At the northern edge of the species distribution successful production of seeds only occurs in years with above-average warm summers; if temperatures fall below 15°C pollen tube growth is inhibited and seeds will not be fertile (Pigott & Huntley, 1981). Vegetative propagation on the other hand is common, forming clonal structures, as *T. cordata* sprouts with great vigour when coppiced (Pigott, 1989, 1991). Trees also spread from

fallen trunks or branches touching the ground that may become rooted and produce new vertical shoots (E.O. Erichsen *pers. obs.*). A pilot study by Hansen et al. (2014) studied individuals from seven populations of *T. cordata* in western Denmark and found abundant clonal structures in two of them. Clonal structures have also been noticed but not specifically analysed in Britain (Logan, Phuekvilai, & Wolff, 2015). They found about 25 per cent of the trees in a typical stand to belong to clonal groups. Recently clonal structures in *Tilia* spp. have been under review in northern Europe where a trend towards more clonality in the leading range-edge populations than in centre populations was found (Logan, Phuekvilai, Sanderson, & Wolff, 2019).

Many plant species employ a mix of clonal and sexual reproduction (Ellstrand & Roose, 1987; Stuefer, Van Hulzen, & During, 2002) as a strategy to survive when environmental conditions are not optimal (Honnay & Bossuyt, 2005). However, clonality may be disadvantageous if the population becomes too homogeneous genetically and, therefore, making the population susceptible to biotic stress and pathogens due to the lack of adaptive potential (Hamilton, 1980). Guerrilla based growth of the clonal structures, where genets are intermixed, as opposed to phalanx where the genet form a tight-packed front of ramets, helps to maintain sexual reproduction as the genotypes are intermixed (Doust, 1981). Clonal populations are likely to preserve genetic variation as the asexual reproduction decreases the impact from genetic drift (Delmotte, Leterme, Gauthier, Rispe, & Simon, 2002; Judson & Normark, 1996). Under the assumption that a decrease in heterozygosity of neutral markers reflects a decrease in variation in genes relevant for performance, it can serve as a proxy for inbreeding depression (Hedrick, 1999; Sunnucks, 2000; Tsitrone, Rousset, & David, 2001).

The fragmented population structure of *T. cordata* and poor ability to establish in new areas gives rise to the hypothesis that the populations may have been isolated and, therefore, prone to genetic drift over a long time. Fragmentation generally has a negative impact on genetic diversity; especially for species with less mobile pollinators (Lowe, Cavers, Boshier, Breed, & Hollingsworth, 2015). Due to being one of the few Danish insect pollinated forest tree species and to the earlier widespread distribution, considerable biodiversity values are associated with the species (Pigott, 1991). Also, with the anticipated climatic change (IPCC, 2014), where Denmark will experience a warmer climate (Olesen et al., 2014), the species may again become more widespread where it will be allowed to spread. This could be in close-to-nature forest management systems, where natural regeneration is a key component and under which all Danish public forests should be managed

(Larsen, 2012). If this happens, knowledge about the amount of genetic variation in the remnant Danish population of Tilia will be highly valuable, as it is this gene pool that a future and potentially larger and more widespread population should build upon.

In the present study, the overall aim is to investigate the population genetic structure of *T. cordata* in Denmark and to which extent the populations contain clonal structures. Hereby we want to increase the knowledge of *T. cordata* populations in northern range-edge populations exemplified in Denmark. More specifically, the study has two concrete objectives. Firstly, to characterise the population genetic structure of *T. cordata* in Denmark with regard to the diversity and differentiation of populations; i.e. to what extent fragmentation of the populations has had an impact on the gene pool and left signs of inbreeding. Secondly, to take a deeper look into the clonal structures and the reproductive patterns found in *T. cordata* populations in Denmark; this is achieved by exhaustive sampling in a selection of populations.

Materials and Methods

Study sites and sampling methods

In total 774 trees were sampled from nine populations, mainly across southern Denmark (Fig. 1). All nine populations are considered to be of ancient or natural origin and with, for the species in Denmark, a substantial number of trees (Lawesson, 2004; Wicksell, 1998). The majority of the populations are located on glacial till with loamy texture, only Draved is located to the west of the Late Weichselian glacial maximums western border where parent material of the soil is glaciofluvial sand populations (Houmark-Nielsen, 1989). Two of the populations were exhaustively sampled and another two were partly exhaustively sampled. In the five remaining populations (Hamborgskoven, Frejlev, Aasen, Aabybjerg and Draved) samples were taken from 32 to 48 trees spread across the populations (stratified sampling; Table 1) – with trees often standing in smaller groups and where the typical distance between sampled trees being >10m, while groups could be more than 100 metres apart. Only trees with a minimum of 2 cm in diameter at breast height (DBH) were included in the study. The sites where exhaustively and partly exhaustively sampling was carried out are characterised as areas where *T. cordata* is dominating in the crown layer. The population Lindoe is from a small peninsula in the lake Røgballe, with no risk of edge effects. For Bolderslev South, Bolderslev North (both sub-populations in the Bolderslev site) and Jonstrupvang sampling was continued until no more neighbours of *T. cordata* were found, and the patch was

hereby considered to be fully sampled. In addition to the areas in Bolderslev that were partly exhaustively sampled, two dykes from the southern and northern part of the forest were also sampled, as well as a small group of *T. cordata* in the north. In Vindeholme sampling was done in two neighbouring stands with different characteristics with regards to DBH, stem number and height. As these relatively large stands consist solely of *T. cordata*, sampling was limited to one corner of each of the two stands.

Stand history for the exhaustively sampled populations

Lindoe, Bolderslev, and Jonstrupvang are all locations with no silvicultural activities today whereas Vindeholme is a production forest with *T. cordata* as the main species. Looking at the first known historical description of Jonstrupvang from 1660 the area was fenced as the King's hunting ground with only limited access to the public. When the land was handed over to the state in the late-mid 19th century it was reported that it was difficult to sell the timber as *T. cordata* was of no interest (Worsøe, 1988). Both Bolderslev and Lindoe show signs of former disturbances. Bolderslev was used for primarily firewood production for local farmers from the mid-18th century, up through the end of the 20th century (Skov- og Naturstyrelsen, 1998). This may explain the absence of large trees in Bolderslev as there was a large demand for wood after WWI and WWII. Lindoe was under protection from 1957 (Overfredningsnævnet, 1957). However, decaying stumps, seen while sampling for this study, showed that some cuttings have been carried out rather late, likely in 2005, before the appointment as forest reserve as it is today.

Growth and spatial data

Leaves were collected for DNA extraction in the autumn 2015 and DBH of sampled trees was measured on the same occasion. Both *T. cordata* and *T. platyphyllos* were sampled as it can be difficult to distinguish the two species in the field. For the exhaustively and partly exhaustively sampled populations, trees were marked for later identification. In spring 2016, before flushing, the three populations Bolderslev, Vindeholme and Lindoe were revisited to obtain spatial information of the sampled trees. To obtain precise information of location for all the trees, coordinates were measured manually. This was done by letting a GPS Trimble XP pro (Trimble, USA) receive approx. 1000 signals on position and use this point as a centre point. From this centre point the distance and bearing to each tree were measured and, the coordinates were calculated. Within clone groups, internal distances among all ramets were measured with an ultrasound measurer (Vertex VI, Haglöfs, Sweden).

Nuclear microsatellites

DNA was extracted from the sampled leaf material using the 96 format kit from Qiagen (Germany). Approximately 40 µg of fresh leave was used and the step-by-step procedure was followed. Genotyping was conducted using ten polymorphic microsatellites (SSRs) developed by Phuekvilai & Wolff (2013). One microsatellite, Tc23, which is not included in the final publication by Phuekvilai & Wolff (2013), was used in this current study. Tc23 was also used with success by (Hansen, Thomsen, & Rasmussen, 2014). The microsatellites were genotyped by multiplexing, using three different primer mixes: mix 1 containing the SSRs Tc6, Tc23, Tc918, Tc920 and Tc937, mix 2 containing the SSRs Tc915, Tc927 and Tc963 and mix 3 containing the SSRs Tc5 and Tc951. Tc918 amplifies in *T. platyphyllos* and the hybrid *T.* x *europaea* but not in *T. cordata* and has consequently been used for species identification (Phuekvilai & Wolff 2013), but was subsequently not included in the population genetic data analyses in the present study. Different master mixes were used for PCR amplification of the three primer mixes. For mix 1 the Qiagen multiplex PCR kit was used, and for mix 2 and 3 the Type-it Microsatellite PCR kit was used (both from Qiagen, Germany). DNA template was diluted 10 times and 0.5 µl of the diluted DNA template was used in the PCR for all 3 mixes. The protocol for amplification followed Phuekvilai & Wolff (2013).

Visualisation of the amplification products from the PCR was done by capillary electrophoresis on an ABI3130xl sequencer (Applied Biosystems). The PCR product was diluted 80 times for mix 1, 130 times for mix 2 and 180 times for mix 3. The size standard GZ500LIZ was used. Data were scored using GeneMapper 4.0 (Applied Biosystems).

Identification of Clones

Clones were identified using Cervus (Kalinowski, Taper, & Marshall, 2007; Marshall, Slate, Kruuk, & Pemberton, 1998). In cases where just one allele in one locus was different between two individuals, they were treated as identical. Since just one allele difference it can either be an error during genotyping or could have been due to a somatic mutation. To make sure that clones are not a product of sexual reproduction, the probability that two unrelated individuals by chance have the same genotype was calculated in GenAlEx 6.5 (Peakall & Smouse, 2012).

Population genetics

In the following analyses only individuals of *T. cordata*, which constituted around 94% of the total number of sampled trees (see results), were included.

Genetic diversity in the nine populations was estimated as the number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity and the inbreeding coefficient (F), all obtained with GenAlEx v6.5 (Peakall & Smouse, 2012). Correction from varying sample sizes in estimates for N_a was done via rarefaction with the software HP-rare (Kalinowski, 2005), where number of genes were set to the lowest number of samples (48 in Aasen).

Genetic differentiation among populations was estimated using both the classic F_{ST} (Wright, 1943) as well as the newer G''_{ST} (Meirmans & Hedrick, 2011). F_{ST} underestimates the differentiation between populations when calculated from markers with a high diversity, such as microsatellites (Hedrick 1999; 2005). However, former F_{ST} was included in order to facilitate comparison with earlier studies where F_{ST} often has been used. Estimation was performed in GenAlEx 6.5 with 9999 permutations of pairwise F_{ST} and G''_{ST} values in order to asses statistical significance. A Mantel test was performed in R version 3.4.2 (packages: ade4, mantel.rtest), based on a Monte Carlo test with 9999 replications.

To test for genetic structure across populations we used a Bayesian clustering algorithm implemented in STRUCTURE 2.2 (Pritchard, Stephens, & Donnelly, 2000). We ran an admixture model with correlated allele frequencies among populations. Five runs were performed for each number of clusters (K: 1-9) with a burn-in of 50,000 and a run length of 100,000 iterations. The web-based version of Pophelper (Francis, 2016) was used to evaluate the STRUCTURE results and to determinate the best number of clusters using the Evanno method (Evanno, Regnaut, & Goudet, 2005).

To see if there were differences in heterozygosity and allele frequency between clonal and non-clonal genotypes, samples were divided into two groups in the Lindoe population and in the two exhaustively sampled areas in Bolderslev and then run in GenAlEx 6.5 using the same procedures as described previously for all the samples.

Clonality and Spatial Genetic Structure

We assume that unique genotypes are a product of sexual reproduction. However, it could also be a last remaining ramet of a pre-existing clonal group and the number of clone groups detected this

way is therefore considered to be a minimum. To characterize the clonal structures in three of the exhaustively sampled areas we took three different dimensions into account: the spatial distribution, the genotypic diversity and the physical dimension (DBH) of the individual trees. For the genotypic diversity, we used the genotypic richness R. Clonality in each population was characterised by a measure of genotypic richness R = (G-1)/(n-1), where n is the number of sampled ramets and G the number of Genets (Dorken & Eckert, 2001). R varies from 0, when all samples have the same genotype, to 1, when all samples have a different genotype. This gives an estimate of the proportion of individuals that are distinguishable and a basis for comparison across populations.

For the populations, Lindoe, Bolderslev South and Bolderslev North analyses of within-population spatial structures were performed by using the mingling index. The mingling index is a calculation of the probability that two neighbours have the same genotype by taking spatial information into account (Pommerening, 2002). The mingling index indicates the degree of clustering of the clones and if the clonal growth belongs to a phalanx strategy, where the species produce a tight-packed group of ramets, or a guerrilla strategy, where ramets are widely spaced and genet stands mixed (Doust, 1981). The mingling index gives the proportion of n=4 nearest neighbours *j* of the *i*th reference tree, where *j*=1 if it does not belong to the same genotype as *i*, and otherwise 0. This gives five potential values for each tree (0, 0.25, 0.5, 0.75 and 1) and the mingling index for the population is then the average of these values for individual trees. The mingling index was calculated in R version 3.4.2, with the package vegan. Finally, the spatial extent of the clone groups was estimated by finding the centre point for clone groups consisting of three ramets or more and then measuring the distance of the ramets to the centre.

Results

Out of the 774 sampled trees, 730 were *T. cordata*, while 44 *T. platyphyllos* or the hybrid *T.* x *europeae* were found in 5 out of 9 populations (Table 2). The 730 *T. cordata* trees contained 485 different genotypes, of which 104 constituted clone groups. The largest clone group was found in Bolderslev North and consisted of 27 ramets. However, the majority of clone groups (46 out of 104) consisted of just two ramets (Table 2). The probability of identity with the nine loci was 2.7×10^{-08}. If only one allele diverged between genotypes the individuals were considered to be identical – assuming the difference being due to mutation. When testing for Hardy-Weinberg equilibrium (HWE), in general, no indication for a deviation from HWE of the loci was seen. However, in

Hamborgskoven, only two out of the nine loci were in HWE and for Bolderslev, it was four out of nine (data not shown).

Genetic diversity

The nine used SSRs showed numbers of alleles per locus ranging from 5 (Tc951) to 32 (Tc963) with an average of 15 alleles per locus (Table 3). Private alleles were found in all populations except Draved (data not shown). The highest rarefracted number of alleles (Na-rare) was seen in Frejlev (7.68) and Bolderslev (7.40), and the lowest was found in Draved (5.75). Generally, the differences in allelic richness among populations are small. The same is seen for H_E where the variation among the nine populations is also limited, ranging from 0.59 in Draved and Lindoe to 0.65 in Frejlev. No noticeable divergence between H_O and H_E was observed. With regard to the fixation index, the only values noticeably different from zero are negative, and therefore there is no indication of a raised amount of homozygotes and inbreeding (-0.06 in Aasen and -0.09 in Aabybjerg) (Table 3).

Separate estimates of H_O and H_E for clones and non-clones show a consistent trend where H_O for clones is higher than H_E leading to a negative fixation index (F) (Table 4). For diversity measures, such as number of alleles, there are no differences between the clonal and non-clonal genotypes (Table 4).

Genetic differentiation

The smallest differentiation estimated with F_{ST} (significance level 0.001) was observed between Bolderslev-Frejlev and Hamborgskoven-Vindeholme, both with $F_{ST} = 0.015$. For G''_{ST} the lowest significant estimate was 0.023 between Frejlev and Hamborgskoven (Table 5). Frejlev, Hamborgskoven and Vindeholme are all on the island Lolland while Bolderslev is located in southern Jutland, relatively far from the three aforementioned populations (Fig. 1). The largest differentiation was found between Draved and Aabybjerg placed in southern and northern Jutland, respectively (F_{ST} 0.046 and G''_{ST} 0.195). The overall F_{ST} was 0.034 and G''_{ST} was 0.103 across populations (P=<0.001). In general, Draved was the population most differentiated from the rest, notwithstanding which population it was compared with. Even Bolderslev, located only 24 km from Draved, was less differentiated from Aabybjerg (G''_{ST} 0.083) 247 km away, than from Draved (G''_{ST} 0.120). The lack of geographically explained genetic differentiation was confirmed by the non-significant Mantel test, with r= 0.397 (P=0.1025) and r=0.411 (P=0.801) for F_{ST} and G''_{ST},

respectively**.** Results from the STRUCTURE analysis did not show any clear clustering of individuals (supplementary materials).

Clonality and spatial structures

Clonal structures were found in all but one population, namely Draved. Genotypic richness R ranged from 0.32 in Bolderslev North to 0.96 in Jonstrupvang (Table 6). The number of ramets per clone is likewise lowest in Jonstrupvang and highest in Bolderslev North where the largest clone group was also found (consisting of 27 ramets).

An analysis of the spatial structures of clone groups and the impact they have on the population structure was done for Lindoe, and for the two sub-populations in Bolderslev (North and South). Jonstrupvang and Vindeholme were left out because of few clonal structures and also Vindeholme because of an expected large edge effect. The mingling index showed a range from 0.47 to 0.58, indicating a quite similar distribution of clones in the three populations (Table 7). However, when looking at the distribution between the five diameter classes it is evident that Bolderslev North is substantially different from the two other populations with only 5 % of the trees in the middle group (mingling class 0.5 - where two neighbours are different and two the same genotype) and approx. 10 per cent-point more trees having no neighbours with different genotypes than in Bolderslev South and Lindoe (Fig. 2). This is likely a result of the large genet consisting of 27 ramets (out of 58 trees in total) in Bolderslev North.

The estimate of the distance from ramet to centre-point in a genet (with minimum 3 ramets) shows that clonal groups in Bolderslev cover larger areas than those in Lindoe, with an average distance of 5.7 meters and 7.1 meters in Bolderslev North and South, respectively, and only 3.2 meters in Lindoe (table 7). One genet in Bolderslev South, consisting of 13 ramets, had an average distance from ramet to centre of 21.7 meters and it is also in this clone group that the largest distance (44.7 meters) was seen. The smallest spatial distribution of the stand was seen in Lindoe where the average was 2.8 meters and the maximum distance from centre of the clone group to a ramet was 7.8 meters. A map of the clones' spatial distribution in Bolderslev North is shown in Figure 3.

The distribution of DBH classes in Lindoe and Bolderslev South and North showed both clonal and non-clonal genets across the whole size range (Fig. 4). In Lindoe and Bolderslev North the largest tree was non-clonal and in Bolderslev South it was a clonal genet. In Bolderslev North and South there were only two trees with DBH larger than 50 cm, or approx. 1.1 per cent of the stand, whereas

5 per cent of the stand in Lindoe exceeded 50 cm in DBH. The largest proportion of big trees was found in Jonstrupvang, where 14 out of 80 trees had a DBH above 80 cm and 31.25 per cent exceeded 50 cm DBH.

Discussion

Genetic analysis of 730 trees including 484 genotypes of *T. cordata* across nine Danish populations showed a gene pool with high diversity among individuals and low differentiation between populations. In most of the populations abundant clonal structures were observed, but in one population clonal structures were entirely absent.

Genetic diversity and differentiation

Tilia cordata showed a high degree of genetic diversity with small differences in the diversity measures across the populations with no indication of inbreeding. This is in accordance with findings from Phuekvilai (2014) that also included populations from Bolderslev and Aabybjerg in their analysis of genetic diversity in *T. cordata* across Europe. Here the genetic diversity in both Danish populations was found to be around the average of all the sampled populations (Phuekvilai 2014; table 4.4 p. 64).

There was no difference between expected and observed heterozygosity, which confirms that. *T. cordata* is an outcrossing species, and there are no signs of inbreeding. It has been suggested that clonality may result in a population excess in heterozygosity (Balloux, Lehmann, & De Meeûs, 2003; Delmotte et al., 2002; Stoeckel et al., 2006) and preserving genetic diversity, as loss of genetic diversity through genetic drift is a process associated with sexual propagation. Comparing clonal genotypes with non-clonal genotypes in three of the populations, a trend formed that the observed heterozygosity is higher than the expected heterozygosity in the clonal genotypes, which supports the idea that clonal growth maintains or even increases heterozygosity levels (Delmotte et al., 2002; Judson & Normark, 1996).

From the pairwise G''_{ST} (and F_{ST}) values we see only a modest differentiation among the populations with an overall G''_{ST} value of 0.103 and pairwise values ranging from 0.016 (Vindeholme-Frejlev) to 0.196 (Draved-Aabybjerg). Draved forest is the only population that differs slightly from the rest - pairwise G''_{ST} values have an average of 0.143, compared to the overall average of 0.103. It is, however, also the only population with no private alleles. One explanation for this pattern, where the differentiation is based solely on different allele frequency

distributions, could be a founder effect followed by limited gene flow, and Draved is, in fact, the population with the lowest diversity, although the differences to the remaining populations are small. Draved was an isolated forest up until modern times placed on an island of clayey till of Saalian time covered with sand dunes and there were large bogs around the forest and the sandy soils were mainly covered with heathland (Møller & Bradshaw, 2001). Ecological isolation could therefore potentially be an explanation of why Draved is more differentiated from the rest of the populations as the environment has prevented neighbouring forests to arise, and thereby limited the gene flow. In a common garden experiment, Lobo et al.(2018) found phenology to follow ecoregions, suggesting isolation by environment to be the main driver structuring the populations. They also genotyped the individuals from the common garden experiment and as in our study, only found limited differentiation among populations in the genetic markers. In a study covering most of Europe Phuekvilai (2014) found indications of isolation by distance in both *T. cordata* and *T. platyphyllos*, latitudinal, and with more genetic diversity in the south.

Despite a long period of fragmentation in the Danish populations of *T. cordata*, there is limited genetic differentiation. This could be due to the fact that the fragmentation of the populations is simply recent compared to the long lifetime of the trees (Hamrick, 2004). *T. cordata* trees can live for >450 years (Pigott 2012) and counting in clonal growth thousands of years is not impossible. All of Denmark is believed to have been covered by forest, with *Tilia spp* as dominant species, and what we find today are remnant subpopulations of the very same former population. Generation time in forest trees is generally high, and especially in *T. cordata* where trees do not produce seeds until they reach the crown layer as heat from the sun is needed for seeds to mature (Tal, 2011). Furthermore, as mentioned above, clonal reproduction may also have prevented genetic drift; a process which causes loss of genetic variation, but also genetically differentiates subpopulations with low or no connectivity through gene flow (Delmotte et al., 2002; Judson & Normark, 1996).

In accordance with the low genetic differentiation, we did not find any clear clustering of the Danish populations of *T. cordata*, resulting in a very weak assignment of populations to different clusters in the STRUCTURE analysis, see supplementary material Fig S2. A study of *T. cordata* in Britain covering a larger geographical area likewise only resulted in vague geographic clusters (Logan et al., 2015). Another study by Phuekvilai (2014) on populations across Europe included two of the same populations as used here (Aabybjerg and Bolderslev South). They found slightly higher pairwise F_{ST} values among these two populations (0.046, which was here 0.021); this might

be explained by their use of 13 instead of nine SSRs (Phuekvilai, 2014). In general, that study also found lower allelic richness (effective number of alleles) - presumably as a function of a larger sample size in our study (Gorman & Renzi, 1979; Leberg, 2002).

Malus sylvestris, the European crab apple, is another of the few insect pollinated tree species native to Denmark and with a similar fragmented distribution. It is mainly found in shelters and in the forest edge and is not a crown bearing tree as *T. cordata*. A study by Larsen et al. (2006) estimated a higher genetic diversity expressed by H_E (0.78) but a similar low differentiation between populations suggesting a high gene flow despite fragmentation.

***How widespread is clonality*?**

We found clones in eight out of nine populations and substantial clonal structures in three out of four exhaustive or partly exhaustively sampled populations. In the two Bolderslev populations (North and South) and Lindoe, the genotypic richness R was 0.32, 0.37 and 0.45, respectively, whereas, in Jonstrupvang R was only 0.96.

The average DBH in the above mentioned four populations, that today has been taken out of active silvicultural management, correlates with the respective genotypic richness R (R^2=0.985, p=0.007, data not shown) so the populations with the largest trees are the populations with the least presence of clonal propagation. As this result is only based on four populations conclusions cannot be directly drawn, but according to Pigott (1989), *T. cordata* only produces basal sprouts when the original stem is lost or damaged. Taken together with these findings it might suggest that, though stems maybe lost during natural hazards, clones are catalysed by coppicing or other management interventions, also backed by the known history of the respective populations. Further, flowering in *T. cordata* is dramatically reduced when coppiced (Waller, Grant, & Bunting, 2012) which promotes vegetatively propagation and thereby clonal structures in the stand. This suggests that absence of clonal growth in a *T. cordata* population may indicate apopulation where very little management has been carried out. This could also explain why no clones were found in Draved forest as that has been without management since 1948 and prior to this only under extensive management, mainly by grazing, up until the 18th century and coppicing, which has been mentioned in management plans in the 19th century (Bradshaw, Wolf, & Møller, 2005). If exhaustively sampling was performed in Draved genotyping may reveal clonal structures, but it seems unlikely that it would be as extensive as in Bolderslev and Lindoe.

Presence of non-clonal trees in all size classes suggests that sexual reproduction, though rare in some populations, is still an active recruitments mechanism in *T. cordata* and the species should be considered as partially clonal in Denmark. In a review of *Tilia* spp. vegetative reproduction is reported to be more frequent than sexual reproduction, and especially so for *T. cordata* (Radoglou et al. 2009, and references therein). However, this is based on morphological observation. Logan et al (2019) did not confirm this, and showed that reproduction is largely sexual, confirming our findings in the Danish populations.

Clonal growth is also known for other species of *Tilia*. *Tilia sibirica*, a close relative to *T. cordata* (Pigott 2012), that only grows in scattered populations in southern Siberia was studied by Logan, Chytrý & Wolff (2018). They sampled at five locations keeping a distance of at least 10 meters between sampled trees and taking approx. 20 trees from each location, and found the genotypic richness to be R=0.601 as a mean over the five populations. They noted that the forest floor was densely overgrown with tall forbs making it hard for regeneration to break through. The American species *T. americana* var *caroliniana* has also shown clonal growth. Evans & Morris (2016) studied two island populations over a period of ten years - observing that the recruitment of new stems was primarily through basal sprouting. Some seedlings did establish, but they did not survive for long enough to grow into larger size classes. Despite this observation, they found the genotypic richness to be 1 and 0.8 respectively on the two islands, indicating little or no clonal growth. However, only a total of 70 individuals were used for genotyping and it is unclear how the sampling of trees was done (e.g. exhaustive or stratified sampling). Both studies show larger genotypic richness, meaning less clonal growth than we found in the populations Lindoe and Bolderslev North and South, we believe this to be primarily a consequence of different sampling methods; expecting the genotypic richness to drop in the T. *sibirica* and *T. americana* var *caroliniana* populations if all neighbouring trees are genotyped. However, it is clear that even though much of the regeneration might be clonal, variation in genotypes is still preserved and studies of *Vaccinium* shrubs shows that even low rates of genet input into long-lived clonal populations can maintain genetic diversity over long periods (Eriksson & Fröborg, 1996; Persson & Gustavsson, 2001).

Stand structures expressed by the mingling index showed that clonal growth in *T. cordata* are acting more as a guerrilla based than a phalanx based growth strategy as the different genotypes are standing intermixed, ensuring gene flow among the genets and enabling relative high levels of genetic diversity. No other studies of *Tilia* spp. has used exhaustively sampling (see below) but similar findings have been seen in *Prunus avium* (Vaughan, Cottrell, Moodley, Connolly, &

Russell, 2007) and *Sorbus torminalis* (Hoebee, Menn, Rotach, Finkeldey, & Holderegger, 2006) where self-incompatibility and mixed genotypes were suggested to effectively reduce the impact of clonality upon sexual reproduction in *S. torminalis* and this was also found to be more pronounced in the unmanaged than in the managed stand of *P. avium*. Generally, we found less clonality in the populations of *T. cordata* than what was reported for both *P. avium* and *S. torminalis* where genotypic richness for *P. avium* was estimated to R=0.286 in the unmanaged stand and R=0.416 in the managed, so more clonal structures in the unmanaged site opposite of what we find here in *T. cordata*. Another study of *S. torminalis* including Danish populations found the clonal reproduction to range from 94 % to 100 % (Kjørup & Kollmann, 2008) which they suggest to be result of very small populations on the northern edge of the distribution.

Conclusions

The present study showed that Danish wild living populations of *T. cordata*, when analysed with neutral nuclear DNA markers, have a high level of genetic diversity, low differentiation among populations and no sign of inbreeding. This is despite the species having a highly fragmented distribution with a presumed low gene flow among populations. Should the changing climate, giving warmer summers, expand the populations of *T. cordata*, the gene pool is in a state that indicates no risk for future inbreeding depression.

This indicates a robust gene pool with no risk for future inbreeding depression and ample adaptive power should the populations of *T. cordata* expand due to warmer summers associated with a changing climate.

The species reproduces both sexually and by clonal propagation. However, currently today in most of the Danish populationsclonal propagation is the primary means of recruitment (Bolderslev North, South and Lindoe) and that clonality may help to maintain heterozygosity in the stands. Furthermore, the clonal structures in *T. cordata* are, when present, adapted towards a guerrilla strategy where genotypes are intermixed ensuring a mix of genotypes when sexual reproduction occurs. Although further research is need our data indicate that clonal structures are affected by stand management history and correlate positively with the amount of disturbance,.

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References

Balloux, F., Lehmann, L., & De Meeûs, T. (2003). The population genetics of clonal and partially clonal diploids. *Genetics*, *164*(4), 1635–1644. https://doi.org/10.1016/j.tree.2005.01.001

Bradshaw, R. H. W., Wolf, A., & Møller, P. F. (2005). Long-term succession in a Danish temperate deciduous forest. *Ecography*, *28*(2), 157–164. https://doi.org/10.1111/j.0906-7590.2005.03980.x

Delmotte, F., Leterme, N., Gauthier, J.-P., Rispe, C., & Simon, J.-C. (2002). Genetic architecture of sexual and asexual populations of the aphid Rhopalosiphum padi based on allozyme and microsatellite markers. *Molecular Ecology*, *11*(4), 711–723. https://doi.org/10.1046/j.1365-294X.2002.01478.x

Dorken, M. E., & Eckert, C. G. (2001). in northern Severely reduced sexual reproduction of a clonal plant , Decodon verticillatus populations (Lythraceae). *Journal of Ecology*, *89*(3), 339–350. https://doi.org/10.1046/j.1365-2745.2001.00558.x

Doust, L. L. (1981). Population dynamics and local specialization in a clonal perrennial (Ranunculus repens): *Journal of Ecology*, *69*, 743–755.

Ellstrand, N. C., & Roose, M. L. (1987). Patterns of Genotypic Diversity in Clonal Plant Species. *American Journal of Botany*, *74*(1), 123–131. https://doi.org/10.1007/sl2229-008-9001-0

Eriksson, O., & Fröborg, H. (1996). "Windows of opportunity" for recruitment in long-lived clonal plants: experimental studies of seedling establishment in Vaccinium shrubs. *Canadian Journal of Botany*, *74*, 1369–1374. https://doi.org/10.1139/b96-166

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Evans, J. P., & Morris, A. B. (2016). Isolated coastal populations of Tilia americana var. Caroliniana persist long-term through vegetative growth. *American Journal of Botany*, *103*(9), 1687–1693. https://doi.org/10.3732/ajb.1600233

Francis, R. M. (2016). pophelper: An r package and web app to analyse and visualize population structure. *Molecular Ecology Resources*, 27–32. https://doi.org/10.1111/1755-0998.12509

Gorman, G. C., & Renzi, J. (1979). Genetic Distance and Heterozygosity Estimates in Electrophoretic Studies : Effects of Sample Size. *Copeia*, *1979*(2), 242–249.

Hamilton, W. D. (1980). Nordic Society Oikos Sex versus Non-Sex versus Parasite. *Oikos*, *35*(2), 282–290.

Hamrick, J. L. (2004). Response of forest trees to global environmental changes. *Forest Ecology and Management*, *197*, 323–335. https://doi.org/10.1016/j.foreco.2004.05.023

Hansen, O. K., Hansen, L. N., Kjær, E. D., & Møller, P. F. (2014). Ny indsigt i de gamle jyske linde - opnået via DNA-metoder. In *40 år for træerne : jubilæumsskrift* (pp. 34–43). København: Dansk Dendrologisk Forening.

Hansen, O. K., Thomsen, P., & Rasmussen, C. W. (2014). DNA markers provide insight about common lime in historical plantings - An example from the Royal Danish Gardens. *Urban Forestry and Urban Greening*, *13*(3), 543–552. https://doi.org/10.1016/j.ufug.2014.04.001

Hedrick, P. W. (1999). Perspective: highly variable loci and their interpretation in evolution and conservation. *International Journal of Organic Evolution*, *53*(2), 313–318.

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution; International Journal of Organic Evolution*, *59*(8), 1633–1638. https://doi.org/Doi 10.1554/05-076.1

Hermy, M., Honnay, O., & Firbank, L. (1999). An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biological Conservation*, *91*, 9–22.

Hoebee, S. E., Menn, C., Rotach, P., Finkeldey, R., & Holderegger, R. (2006). Spatial genetic structure of Sorbus torminalis: The extent of clonal reproduction in natural stands of a rare tree species with a scattered distribution. *Forest Ecology and Management*, *226*(1–3), 1–8. https://doi.org/10.1016/j.foreco.2005.12.024

Honnay, O., & Bossuyt, B. (2005). Prolonged clonal growth: escape route or route to extinction? *Oikos*, *108*(2), 427–432. https://doi.org/10.1111/j.0030-1299.2005.13569.x

512 Houmark-Nielsen, M. (1989). The Last Interglacial-Glacial Cycle in Denmark. *Quaternary*
513 *International*, *3/4*, 31–39.

514 IPCC. (2014). *Climate Change 2014: Synthesis Report*. *Contribution of Working Groups I, II and*
515 *III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Geneva,
516 Switzerland.

517 Judson, O. P., & Normark, B. B. (1996). Ancient asexual scandals. *Trends in Ecology and*
518 *Evolution*, *11*(2 SPEC. ISS.), 41–46. https://doi.org/10.1016/0169-5347(96)81040-8

519 Kalinowski, S. T. (2005). HP-RARE 1.0: A computer program for performing rarefaction on
520 measures of allelic richness. *Molecular Ecology Notes*, *5*(1), 187–189.
521 https://doi.org/10.1111/j.1471-8286.2004.00845.x

522 Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program
523 cervus accommodates genotyping error increases success in paternity assignment. *Molecular*
524 *Ecology*, *16*(5), 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

525 Kjørup, K., & Kollmann, J. (2008). Low genetic diversity in small peripheral populations of a rare
526 European tree (Sorbus torminalis) dominated by clonal reproduction. *Conservation Genetics*,
527 (9), 1533–1539. https://doi.org/10.1007/s10592-007-9492-y

528 Larsen, A. S., Asmussen, C. B., Coart, E., Olrik, D. C., & Kjær, E. D. (2006). Hybridization and
529 genetic variation in Danish populations of European crab apple (Malus sylvestris). *Tree*
530 *Genetics & Genomes*, (2), 86–97. https://doi.org/10.1007/s11295-005-0030-0

531 Larsen, J. B. (2012). Close-to-Nature Forest Management: The Danish Approach to Sustainable
532 Forestry. In J. J. Diez & J. M. García (Eds.), *Sustainable Forest Management - Current*
533 *Research* (pp. 199–218). IntechOpen. https://doi.org/10.5772/32009

534 Lawesson, J. E. (2004). Ancient forests in Denmark and the importance of Tilia. *Forest*
535 *Biodiversity: Lessons from History for Conservation*, *10*, 0–97.

536 Leberg, P. L. (2002). Estimating allelic richnes: Effects of sample size and bottlenecks. *Molecular*
537 *Ecology*, *11*, 2445–2449. https://doi.org/10.1046/j.1365-294X.2002.01612.x

538 Lobo, A., Kim, O., Jon, H., Hansen, K., Ortvald, E., Jacobsen, B., & Dahl, E. (2018). Local
539 adaptation through genetic differentiation in highly fragmented Tilia cordata populations,
540 (October 2017), 1–9. https://doi.org/10.1002/ece3.4131

Logan, S. A., Phuekvilai, P., Sanderson, R., & Wolff, K. (2019). Reproductive and population genetic characteristics of leading-edge and central populations of two temperate forest tree species and implications for range expansion. Forest Ecology and Management, 433(November 2018), 475–486. https://doi.org/10.1016/j.foreco.2018.11.024

Logan, S. A., Chytrý, M., & Wolff, K. (2018). Genetic diversity and demographic history of the Siberian lime (Tilia sibirica). *Perspectives in Plant Ecology, Evolution and Systematics*, *33*(July 2017), 9–17. https://doi.org/10.1016/j.ppees.2018.04.005

Logan, S. A., Phuekvilai, P., & Wolff, K. (2015). Ancient woodlands in the limelight: delineation and genetic structure of ancient woodland species Tilia cordata and Tilia platyphyllos (Tiliaceae) in the UK. *Tree Genetics and Genomes*, *11*(3). https://doi.org/10.1007/s11295-015-0872-z

Lowe, A. J., Cavers, S., Boshier, D., Breed, M. F., & Hollingsworth, P. M. (2015). The resilience of forest fragmentation genetics—no longer a paradox—we were just looking in the wrong place. *Heredity*, *115*(2). https://doi.org/10.1038/hdy.2015.40

Marshall, T. C., Slate, J., Kruuk, L. E. B., & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, *7*(5), 639–655. https://doi.org/10.1046/j.1365-294x.1998.00374.x

Meirmans, P. G., & Hedrick, P. W. (2011). Assessing population structure: FST and related measures. *Molecular Ecology Resources*, *11*(1), 5–18. https://doi.org/10.1111/j.1755-0998.2010.02927.x

Møller, P. F., & Bradshaw, R. H. W. (2001). Draved skov- et dansk forskningsprojekt gennem mere end 50 år. Geologi Nyt. GEUS København.

Olesen, M., Madsen, K. S., Ludwigsen, C. A., Boberg, F., Christensen, T., Cappelen, J., … Hesselbjerg Christensen, J. (2014). *Fremtidige klimaforandringer i Danmark. Danmarks Klimacenter rapport*. https://doi.org/978-87-7478-616-0

Overfredningsnævnet. (1957). *Reg. nr.: 02111.00 Fredningen vedrører: Maribo Søerne*.

Peakall, R., & Smouse, P. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics (Oxford, England)*, *1*, 6–8. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Persson, H. A., & Gustavsson, B. A. (2001). The extent of clonality and genetic diversity in

lingonberry (Vaccinium vitis-idaea L.) revealed by RAPDs and leaf-shape analysis. *Molecular Ecology*, *10*(6), 1385–1397. https://doi.org/10.1046/j.1365-294X.2001.01280.x

Phuekvilai, P. (2014). *Relicts, Refugia and Reticulation: A study of Population History, Hybrids and Phylogeny in the Long-Lived Flowering Tree Genus Tilia*. Newcastle University.

Phuekvilai, P., & Wolff, K. (2013). Characterization of Microsatellite Loci in Tilia platyphyllos (Malvaceae) and Cross-Amplification in Related Species. *Applications in Plant Sciences*, *1*(4), 1200386. https://doi.org/10.3732/apps.1200386

Pigott, C. D. (1989). Factors controlling the distribution of Tilia cordata Mill at the northern limits of its geographical range IV . Estimated ages of the trees.

Pigott, C. D. (1991). Tilia Cordata Miller. *The Journal of Ecology*, *79*(4), 1147. https://doi.org/10.2307/2261105

Pigott, C. D. (2012). *Lime-trees and basswoods : a biological monograph of the genus Tilia*. Cambridge University Press.

Pigott, C. D., & Huntley, J. P. (1981). Factors controlling the distribution of Tilia cordata at its northern limit of its geographical limits. III. Nature and Causes of Seed Sterility. *New Phytologist*, *87*(4), 817–839. https://doi.org/10.1111/j.1469-8137.1981.tb01716.x

Pommerening, A. (2002). Approaches to quantifying forest structures. *Forestry*, *75*(3), 305–324. https://doi.org/10.1093/forestry/75.3.305

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, *155*(2), 945–59. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Rackham, O. (2008). Ancient woodlands: modern threats. *New Phytologist*, *180*(3), 571–586. https://doi.org/10.1111/j.1469-8137.2008.02579.x

Radoglou, K., Dobrowolska, D., Spyroglou, G., & Nicolescu, V.-N. (2009). A review on the ecology and silviculture of limes: (*Tilia cordata* Mill., *Tilia platyphyllos* Scop, and *Tilia tomentosa* Moench.) in Europe. *Die Bodenkultur*, *60*(3), 9–19. https://doi.org/http://www.valbro.uni-freiburg.de/

Rehfeldt, N. (1994). *Fra Istid til Bøgetid* (2nd ed.). Naturhistorisk museum.

Skov- og Naturstyrelsen. (1998). *Biologiske Undersøgelser Bolderslev og Uge Skov*.

Stoeckel, S., Grange, J., Fernández-Manjarres, J. F., Bilger, I., Frascaria-Lacoste, N., & Mariette, S. (2006). Heterozygote excess in a self-incompatible and partially clonal forest tree species - Prunus avium L. *Molecular Ecology*, *15*(8), 2109–2118. https://doi.org/10.1111/j.1365-294X.2006.02926.x

Stuefer, J. F., Van Hulzen, J. B., & During, H. J. (2002). A genotypic trade-off between the number and size of clonal offspring in the stoloniferous herb Potentilla reptans. *Journal of Evolutionary Biology*, *15*(5), 880–884. https://doi.org/10.1046/j.1420-9101.2002.00435.x

Sunnucks, P. (2000). Efficient genetic markers for population biology. *Trends in Ecology & Evolution*, *15*(5), 199–203.

Tal, O. (2011). Flowering phenological pattern in crowns of four temperate deciduous tree species and its reproductive implications. *Plant Biology*, *13*(SUPPL. 1), 62–70. https://doi.org/10.1111/j.1438-8677.2010.00386.x

Tsitrone, A., Rousset, F., & David, P. (2001). Heterosis, Marker Mutational Processes and Population Inbreeding History. *Genetics*, (159), 1845–1859.

Turner, J. (1962). the Tilia Decline: an Anthropogenic Interpretation. *New Phytologist*, *61*(3), 328–341. https://doi.org/10.1111/j.1469-8137.1962.tb06304.x

Vaughan, S. P., Cottrell, J. E., Moodley, D. J., Connolly, T., & Russell, K. (2007). Clonal structure and recruitment in British wild cherry (Prunus avium L.). *Forest Ecology and Management*, *242*(2–3), 419–430. https://doi.org/10.1016/j.foreco.2007.01.059

Waller, M., Grant, M. J., & Bunting, M. J. (2012). Modern pollen studies from coppiced woodlands and their implications for the detection of woodland management in Holocene pollen records. *Review of Palaeobotany and Palynology*, *187*, 11–28. https://doi.org/10.1016/j.revpalbo.2012.08.008

Wicksell, U. (1998). *En foreløbig opgørelse af Lindeforekomster i Danmark*. Hørsholm.

Worsøe, E. (1988). *Jonstrup Vang Historien om et skovlandskab fra middelalderen til nutid*. Skippershoved.

Wright, S. (1943). Isolation by distance. *Genetics*, *28*, 114–128.

Young, A., Boyle, T., & Brown, T. (1996). The population genetic consequences of habitat fragmentation for plants. *Tree*, *11*(10), 413–418.

Tables

Table 1 Sampling of nine populations across Denmark, with sampling, either by stratified sampling or by exhaustively sampling, indicated. Partly exhaustively sampled refers to populations where all trees have been sampled within a selected part of the forest (sub-populations).

Population	No. of samples	Sub-population	No. of samples	Sampling method	Coordinates
Bolderslev all	286				55°00’04”N 9°21’25”E
		B. South	175	Partly exhaustively sampled	
		B. south dyke	22	Stratified sampling	
		B. North 1	58	Partly exhaustively sampled	
		B. North 2	8	Partly exhaustively sampled	
		B. north dyke	23	Stratified sampling	
Draved	40			Stratified sampling	55°00’51”N 8°57’55”E
Frejlev	32			Stratified sampling	54°41’31”N 11°40’44”E
Hamborgskoven	48			Stratified sampling	54°47’11”N 11°48’28”E
Jonstrupvang	80			Exhaustively sampled	55°45’39”N 12°22’37”E
Aasen	32			Stratified sampling	55°27’54”N 12°09’03”E
Lindoe	147			Exhaustively sampled	54°43’01”N 11°34’30”E
Vindeholme	79			Partly exhaustively sampled	54°44’34”N 11°06’19”E
		V. East (not thinned)	47		
		V. West (thinned)	32		
Aabybjerg	30			Stratified sampling	57°12’33”N 9°44’49”E

Table 2 Number of samples, clones, genotypes and presence of *T. platyphyllos* and *T. x europea* in each population. No. of samples: total amount of trees sampled, *T. platyphyllos* and *T. x europaea:* number of samples where marker Tc918 amplified, Clones: number of genetes that consist of more than one ramet with ramets in brackets the total number of ramets, number of genotypes: number of unique genotypes of *T. cordata*

Population	No. of samples	*T. platyphyllos* and *T. x europaea*	Clones	No. of Genotypes
			Genets (ramets)	*T. cordata*
Bolderslev all	286	19	52 (203)	117
Draved	40	0	0	40
Frejlev	32	2	2 (4)	28
Hamborgskoven	48	8	4 (9)	35
Jonstrupvang	80	0	3 (6)	77
Aasen	32	1	6 (13)	24
Lindoe	147	14	28 (95)	66
Vindeholme	79	0	7 (16)	70
Aabybjerg	30	0	2 (4)	28
All (sum)	*774*	*44*	*104 (350)*	*485*

Table 3 Genetic diversity of T. cordata for each of the nine populations. N is the number of genotypes, Na the number of alleles, Na-rare the rarefacted number of alleles, Ne the effective number of alleles, H_O observed heterozygosity, H_E expected heterozygosity and F the fixation index.

Population	N	Na	Na-rare	Ne	H_O	H_E	F
Bolderslev all	116	10.89	7.40	4.77	0.62	0.64	0.01
Draved	40	6.33	5.75	3.51	0.59	0.59	-0.01
Frejlev	28	8.00	7.68	4.53	0.62	0.65	0.03
Hamborgskoven	35	7.89	7.00	3.82	0.63	0.63	0.01
Jonstrupvang	77	8.44	6.51	3.96	0.61	0.63	0.01
Aasen	24	6.33	6.33	3.66	0.63	0.60	-0.06
Lindoe	66	7.78	6.43	3.80	0.59	0.59	-0.01
Vindeholme	70	7.78	6.21	3.88	0.60	0.61	0.00
Aabybjerg	28	6.78	6.57	4.12	0.68	0.63	-0.09
All	*484*	*7.80*	*6.65*	*4.01*	*0.62*	*0.62*	*-0.01*

Table 4 Genetic diversity separately for clonal and non-clonal genotypes of the populations in Bolderslev, including all sub-populations, and Lindoe. N is the number of genotypes, Na the number of alleles, Na-rare the rarefacted number of alleles, Ne the effective number of alleles, Ho observed heterozygosity, He expected heterozygosity and F the fixation index.

Population		N	Na	Na-rare	Ne	H_O	H_E	F
Lindoe	clone	29	6.44	4.33	3.56	0.61	0.59	-0.03
	non-clone	37	7.22	4.39	3.72	0.57	0.59	0.03
Bolderslev all	clone	42	8.22	4.81	4.52	0.63	0.63	-0.02
	non-clone	74	10.22	4.79	4.60	0.62	0.64	0.02

Table 5 Genetic differentiation among populations, F_{ST} below diagonal and G''_{ST} above. All numbers are significant on a 0.001 level except for the three marked with †

	Bolderslev all	Frejlev	Hamborg-skoven	Jonstrup vang	Lindoe	Vindeholme	Aasen	Aabybjerg	Draved
Bolderslev all	0	0.053	0.083	0.102	0.115	0.081	0.115	0.083	0.120
Frejlev	0.015	0	0.023	0.088	0.103	0.016†	0.096	0.094	0.106
Hamborgskoven	0.020	0.012†	0	0.084	0.108	0.053	0.083	0.079	0.128
Jonstrupvang	0.022	0.022	0.021	0	0.065	0.091	0.091	0.109	0.157
Lindoe	0.025	0.026	0.027	0.016	0	0.104	0.119	0.112	0.141
Vindeholme	0.018	0.009†	0.015	0.021	0.025	0	0.104	0.122	0.151
Aasen	0.028	0.028	0.025	0.024	0.031	0.028	0	0.149	0.149
Aabybjerg	0.021	0.026	0.022	0.026	0.028	0.029	0.038	0	0.195
Draved	0.028	0.028	0.032	0.036	0.034	0.036	0.039	0.046	0

Table 6 Clonality shows the total number of samples in each of the exhaustively sampled populations; sub-samples for Bolderslev North and South, Jonstrupvang, Lindoe and Vindeholme, including information on number of genotypes, no. of *T. cordata* clone groups, and ramet per clone group and genotypic richness (R = (G-1)/(n-1)

Population	No. Of samples	No. of Genotypes *T. cordata*	Clone groups Genets (ramets)	Ramets per clone group	Genotypic richness R
B. South	162	66	30(126)	4.20	0.37
B. North	57	19	8(46)	5.75	0.32
Jonstrupvang	80	77	3(6)	2.00	0.96
Lindoe	133	66	28(95)	3.39	0.45
Vindeholme	79	70	7(16)	2.29	0.88

682 **Table 7** Spatial structures of three exhaustively sampled populations. Calculated mingling index
683 and the spatial distribution of clone groups expressed as distance from centre of a genet to ramets.

	Mingling index	Distance from clone centre [meter] (>2 ramets)	
		Average	max
Lindoe	0.58	3.2	7.4
B. North	0.47	5.7	12.1
B South	0.52	7.1	44.7

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Figure legends

Figure 1 - Map of the nine locations across Denmark, yellow shows the exhaustively sampled sites and blue the rest

Figure 2 Distribution of mingling classes in per cent for the three populations, Bolderslev North, Bolderslev South and Lindoe.

Figure 3 Spatial distribution of trees in sub-population Bolderslev North (55°00’14”N 9°21’25”E), red dots mark trees with unique genotypes and black signs mark the different clonal groups at the site

Figure 4 Diameter distribution shown as size classes in 5 cm intervals for the four exhaustively sampled populations, the y-axis are shown as a percentage of the whole population